

Express Mail: EL521417745US

TRANSMITTAL LETTER TO THE UNITED STATES

DSI-10002/22

DESIGNATED/ELECTED OFFICE (DO/EO/US)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

CONCERNING A FILING UNDER 35 U.S.C. 371

09 / 462950

INTERNATIONAL APPLICATION NO.
PCT/US98/14776INTERNATIONAL FILING DATE
16 July 1998PRIORITY DATE CLAIMED
16 July 1997

TITLE OF INVENTION

METHOD FOR ACQUIRING, STORING AND ANALYZING CRYSTAL IMAGES

520 Rec'd PCT/PTO 14 JAN 2000

APPLICANT(S) FOR DO/EO/US

GESTER, Thomas E. et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

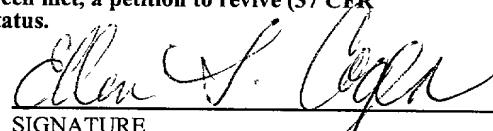
1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. has been transmitted by the International Bureau.
 - c. is not required, as the application was filed in the United States Receiving Office (RO/US).
6. A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. A copy of the International Search Report (PCT/ISA/210).
8. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. have been transmitted by the International Bureau.
 - c. have not been made; however, the time limit for making such amendments has NOT expired.
 - d. have not been made and will not be made.
9. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included:

13. An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. A **FIRST** preliminary amendment.
A SECOND or SUBSEQUENT preliminary amendment.
16. A substitute specification.
17. A change of power of attorney and/or address letter.
18. Certificate of Mailing by Express Mail
19. Other items or information:

Article 34 Amendment dated 2/16/99

Article 34 Amendment dated 7/20/99

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 09/462950	INTERNATIONAL APPLICATION NO. PCT/US98/14776	ATTORNEY'S DOCKET NUMBER DSI-10002/22												
20. The following fees are submitted:														
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :														
<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 80%; vertical-align: top;"> <input type="checkbox"/> Search Report has been prepared by the EPO or JPO \$840.00 <input checked="" type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) \$670.00 <input type="checkbox"/> No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$760.00 <input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$970.00 <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$96.00 </td> <td style="width: 20%; vertical-align: top; text-align: right;"> CALCULATIONS PTO USE ONLY </td> </tr> </table>			<input type="checkbox"/> Search Report has been prepared by the EPO or JPO \$840.00 <input checked="" type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) \$670.00 <input type="checkbox"/> No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$760.00 <input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$970.00 <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$96.00	CALCULATIONS PTO USE ONLY										
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ENTER APPROPRIATE BASIC FEE AMOUNT = \$670.00														
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)). \$0.00														
<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 25%;">CLAIMS</th> <th style="width: 25%;">NUMBER FILED</th> <th style="width: 25%;">NUMBER EXTRA</th> <th style="width: 25%;">RATE</th> </tr> </thead> <tbody> <tr> <td>Total claims</td> <td>9 - 20 =</td> <td>0</td> <td>x \$18.00 \$0.00</td> </tr> <tr> <td>Independent claims</td> <td>1 - 3 =</td> <td>0</td> <td>x \$78.00 \$0.00</td> </tr> </tbody> </table>			CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	Total claims	9 - 20 =	0	x \$18.00 \$0.00	Independent claims	1 - 3 =	0	x \$78.00 \$0.00
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE											
Total claims	9 - 20 =	0	x \$18.00 \$0.00											
Independent claims	1 - 3 =	0	x \$78.00 \$0.00											
Multiple Dependent Claims (check if applicable). <input type="checkbox"/> \$0.00														
TOTAL OF ABOVE CALCULATIONS = \$670.00														
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable). <input checked="" type="checkbox"/> \$335.00														
SUBTOTAL = \$335.00														
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). + \$0.00														
TOTAL NATIONAL FEE = \$335.00														
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). <input type="checkbox"/> \$0.00														
TOTAL FEES ENCLOSED = \$335.00														
Amount to be: refunded \$ charged \$														
<p><input checked="" type="checkbox"/> A check in the amount of \$335.00 to cover the above fees is enclosed.</p> <p><input type="checkbox"/> Please charge my Deposit Account No. 07-1180 in the amount of \$ to cover the above fees. A duplicate copy of this sheet is enclosed.</p> <p><input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 07-1180 A duplicate copy of this sheet is enclosed.</p>														
<p>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</p>														
<p>SEND ALL CORRESPONDENCE TO:</p> <div style="border: 1px solid black; padding: 5px; display: inline-block;"> Ellen S. Cogen Gifford, Krass, Groh, Sprinkle, Anderson & Citkowski, P.C. 280 N. Old Woodward Ave., Ste. 400 Birmingham, MI 48009 (248)647-6000 </div>														
 SIGNATURE														
Ellen S. Cogen NAME														
38,109 REGISTRATION NUMBER														
14 January 2000 DATE														

09/462950
428 Rec'd PCT/PTO 14 JAN 2000

Attorney Docket No. DSI-10052/22

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Diversified Scientific, Inc.

Int'l Application No.: PCT/US98/14776

Filed: 16 July 1998

Priority Date: 16 July 1997

Title: METHOD FOR ACQUIRING, STORING AND ANALYZING CRYSTAL IMAGES

AMENDMENT UNDER RULES 66.3 AND 66.8

Box PCT
Assistant Commissioner of Patents
Washington, DC 20231

Dear Sir:

Substitute pages 16, 16a, and 16b are being submitted containing claims 1-14. The status of these claims are as follows:

Claims 1, 5, 6, 9, 11, 12 and 13 have been amended.

Claims 2, 3, 4, 10 and 14 have been canceled.

Claims 7 and 8 remain unchanged.

The principal amendment is to system claim 1 to clearly define over the art of record.

Claims 1, 2, 5, 8, 10 and 12 were held to lack an inventive step under PCT Article 33(3) as being obvious over Hartley et al. (U.S. 5,544,254).

Independent claim 1 has been amended to clearly define over Hartley by incorporating a claim limitation of prior claims 3, 4 or 14. Prior claims 3, 4 and 14 each satisfying the requirements of PCT Articles 33(2)-(4). As such, it is believed that dependent claims 5 and 8 which depend therefrom similarly define an inventive step now. In view of the system of claim 1, it is submitted that utilizing a movable stage, as opposed to camera movement, represents an inventive step over the prior art of record.

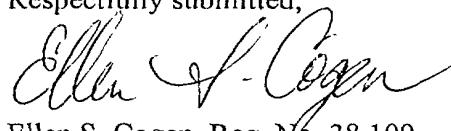
Claims 6, 11 and 13 have been amended into independent form. As such, claims 6, 11 and 13 satisfy the criteria of PCT Article 33(2)-(4).

It is now believed that all the claims define an inventive step which is not obvious over the prior art.

The above amendment does not go beyond the disclosure in the international application as filed.

If the Examiner has any further questions relating to this application, Applicant's attorney may be reached at (248) 647-6000.

Respectfully submitted,



Ellen S. Cogen, Reg. No. 38,109
Gifford, Krass, Groh, Sprinkle,
Anderson & Citkowski, P.C.
280 N. Old Woodward, Suite 400
Birmingham, MI 48009
(248) 647-6000

Dated: 7/9/99

ANG/gs

DEPARTMENT OF COMMERCE
U.S. PATENT AND TRADEMARK OFFICE

Claims

1 1. A system for utilizing a digital computer to evaluate microscopic
2 details of at least one crystal, comprising:
3 a camera which generates an output signal representative of an image
4 positioned in a focal plane of the camera,
5 a tray which positions said at least one crystal in said focal plane,
6 a computer processing unit having a persistent storage device, which
7 computer processing unit acquires said output signal from said camera,
8 said computer processing unit being programmed to evaluate said stored
9 image from said camera and for generating a result signal representative thereof,
10 said computer processing unit being programmed to store said result
11 signals in said persistent storage device and performing at least one function
12 selected from the group consisting of: to count crystals, to generate three-
13 dimensional surface plots of crystals within a database, and to determine crystal
14 size by determination of the length of the perimeter of said crystals.

1 5. The invention as defined in claim 1 and comprising a movable
2 stage for automatically positioning said at least one crystal in said focal plane.

1 6. A system for utilizing a digital computer to evaluate microscopic
2 details of at least one crystal, comprising:
3 a camera which generates an output signal representative of an image
4 positioned in a focal plane of the camera,
5 a tray which positions said at least one crystal in said focal plane,
6 a computer processing unit having a persistent storage device, which
7 computer processing unit acquires said output signal from said camera,
8 said computer processing unit being programmed to evaluate said stored
9 image from said camera and for generating a result signal representative thereof,
10 said computer processing unit being programmed to store said result
11 signals in said persistent storage device, and
12 a T-squared filter to identify said at least one crystal.

1 7. The invention as defined in claim 6 where said T-squared filter
2 comprises a software program executed by said computer processing unit.

1 8. The invention as defined in claim 5 wherein said computer
2 processing unit generates output signals to control the movement of said movable
3 stage.

1 9. The invention as defined in claim 1 wherein data relating to said
2 at least one crystal is stored in the database.

1 11. A system for utilizing a digital computer to evaluate microscopic
2 details of at least one crystal, comprising:

3 a camera which generates an output signal representative of an image
4 positioned in a focal plane of the camera,

5 a tray which positions said at least one crystal in said focal plane,

6 a computer processing unit having a persistent storage device, which
7 computer processing unit acquires said output signal from said camera,

8 said computer processing unit being programmed to evaluate said stored
9 image from said camera and for generating a result signal representative thereof.

10 said computer processing unit being programmed to store said result
11 signals in said persistent storage device, and

12 an optical fiber extending between a light source directed onto said at least
13 one crystal and said at least one crystal.

1 12. The invention as defined in claim 1 and comprising a computer
2 algorithm executed by said computer processing unit for rating said at least one
3 crystal with respect to predetermined standards.

1 13. A system for utilizing a digital computer to evaluate microscopic
2 details of at least one crystal, comprising:

3 a camera which generates an output signal representative of an image
4 positioned in a focal plane of the camera,

5 a tray which positions said at least one crystal in said focal plane,

6 a computer processing unit having a persistent storage device, which
7 computer processing unit acquires said output signal from said camera,

8 said computer processing unit being programmed to evaluate said stored
9 image from said camera and for generating a result signal representative thereof,

10 said computer processing unit being programmed to store said result
11 signals in said persistent storage device, and

12 a computer algorithm executed by said computer processing unit for
13 simulating edges of crystals missing in said image generated by said camera.

09/462950

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PCT

The received stamp of the U.S. Receiving Office imprinted hereon
hereon acknowledges the filing of:

Transmittal Letter PCT Request _____ Page(s)
 Fee Calculation Sheet

 Page(s) Specifications Page(s) Claims
 Page(s) Abstract Sheets of Drawings
 Chapter II Demand Amendment (Article 34) 62 Page(s)
 Invitation to Correct Defects Power of Attorney Check _____

Other: _____

Applicant: Diversified Scientific Inc.

Serial/Reg. No. PCT/US98/14776 Atty Docket No. DSI-10052727
Date: 07/21/99 Date Due: 08/10/99
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**VERIFIED STATEMENT (DECLARATION)
CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(c-f) and 1.27(b-d))**

With respect to the invention by Thomas E. Gester, William M. Rosenblum, Gayle K. Christopher, David T. Hamrick, Lawrence J. DeLucas and Brian Tillotson, entitled METHOD FOR ACQUIRING, STORING AND ANALYZING CRYSTAL IMAGES described in

the specification filed herewith

application no. _____, filed _____

patent no. _____, issued _____

I. IDENTIFICATION OF DECLARANT AND RIGHTS AS A SMALL ENTITY

I hereby declare that I am

(a) Independent Inventor

a below named independent inventor, and that I qualify as an independent inventor, as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under Sections 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office.

(b) Non-inventor Supporting a Claim By Another

making this verified statement to support a claim by _____ for a small entity status for purposes of paying reduced fees under Sections 41(a) and (b) of Title 35, United States Code, and I hereby declare that I would qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under Sections 41(a) and (b) of Title 35, United States Code, if I had made the above identified invention.

(c) Small Business concern

the owner of the small business concern identified below:

an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN Diversified Scientific, Inc.

ADDRESS OF CONCERN 1075 13th St. South, Birmingham, AL 35205-3409

That the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Sections 41(a) and (b) of title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

(d) Non-Profit Organization

an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: _____

ADDRESS OF ORGANIZATION: _____

TYPE OF ORGANIZATION

University or other institution of higher education
 Tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3))
 Nonprofit scientific or educational under Statute of State of the United States of America
(Name of State _____)
(Citation of Statute _____)
 Would qualify as tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)),
if located in the United States of America
 Would qualify as nonprofit scientific or educational under Statute of State of the United States of
America if located in the United States of America
(Name of State _____)
(Citation of Statute _____)

and that the nonprofit organization identified above qualifies as a nonprofit organization as defined
in 37 CFR 19(e) for purposes of paying reduced fees under Sections 41(a) and (b) of Title 35, United States
Code.

II. OWNERSHIP OF INVENTION BY DECLARANT

I hereby declare that rights under contract or law remain with and/or have been conveyed to the above
identified

person (item (a) or (b) above)
 concern (item (c) above)
 organization (item (d) above)

EXCEPT, that if the rights held are not exclusive, each individual, concern having rights to the
invention is listed below and no rights to the invention are held (1) by any person who could not be classified
as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, (2) any concern which
would not qualify as a small business concern under 37 CFR 1.9(d) or (3) a nonprofit organization under 37
CFR 1.9(e)

no such person, concern or organization
 person, concerns or organizations listed below

FULL NAME _____ ADDRESS _____

INDIVIDUAL SMALL BUSINESS CONCERN NONPROFIT ORGANIZATION

III. ACKNOWLEDGEMENT OF DUTY TO NOTIFY PTO OF STATUS CHANGE

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

IV. DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Thomas E. Gester

TITLE OF PERSON President

ADDRESS OF PERSON SIGNING 1075 13th St. South, Birmingham, AL 35205-3409

SIGNATURE TEGester DATE 7/16/97

DSI-10052/22
80716gs

METHOD FOR ACQUIRING, STORING
AND ANALYZING CRYSTAL IMAGES.

This invention was made with Government support under contract NAS8-40839 awarded by NASA. The Government has certain rights in this invention.

5

Background of the Invention

I. Field of the Invention

The present invention relates to a computer controlled system which acquires, stores and analyzes crystal images and other parameters relevant to the crystals, or microscopic details of other specimens.

10

II. Description of the Prior Art

In many chemical, pharmaceutical and medical applications crystals, e.g. protein crystals, are grown in trays for subsequent evaluation by a lab technician or scientist (hereinafter collectively referred to as "technician"). In evaluating the crystals once grown, the lab technician examines the crystals under the microscope and then visually evaluates or rates individual crystals. The straightness of the crystal edges, size of the crystal, presence or absence of flaws in the crystal as well as other crystal parameters are used by the technician in his or her rating process. The technician may also maintain notes of other parameters, such as pH, crystal growing time, temperature, et cetera, which are relevant to the particular crystal.

15

In some instances, the technician will take and maintain a photograph of the particular crystal under examination. The photograph is then stored along with the notes relevant to the particular crystals in the particular tray.

20

This previously known system for evaluating and rating crystals is disadvantageous for a number of reasons. Most prominently, the crystal evaluation and rating system is labor intensive and, thus, not only slow and expensive in labor costs, but also tedious for the technician.

25

A still further disadvantage of these previously known systems is that the photographic record used for the examined crystal is expensive in material costs from the photographic process. Furthermore, photographs by their very nature are easily damaged and also deteriorate over time.

DSI-10052/22
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A still further disadvantage of the previously known method for evaluating and rating crystals is that no efficient means or system has been previously known for cross-referencing the various crystals and crystal parameters relative to each

other. Instead, the photographic picture as well as the other parameters relevant to the particular picture are simply maintained separately from the crystal specimens.

Summary of the Present Invention

5 The present invention provides a system utilizing a digital computer which overcomes all of the above-mentioned disadvantages of acquiring, storing and evaluating crystals, such as protein crystals, or microscopic details of other specimens.

10 In brief, the present invention utilizes a video camera which provides a digital output signal representative of an object, in this case crystals maintained within a tray, positioned within its focal window. Preferably, a central computer controls a movable stage to sequentially position trays in the focal window. The camera is operated under the control of a central computer which not only activates the initiation of the image acquisition by the camera, but also stores the 15 output from the camera in digital form on data storage media. Preferably, the optical image from the video camera is stored on removable data storage media, such as a JAZZ™ drive, ZIP™ drive or CD ROM, etc..

20 In addition to storing the acquired optical image from the video camera, the technician, via a computer keyboard, mouse or other computer input means, also inputs data corresponding to parameters relevant to the particular crystal specimen under examination. Such parameters can include, for example, the pH, temperature, duration of crystal growth, et cetera for the particular crystal specimen. Additionally, the computer software preferably provides input template 25 configurations to simplify the data input of the parameters by the technician and thus avoid or at least minimize the redundant input of information for different trays having similar parameters.

30 The various stored parameters may also be stored with the optical image on the data storage media. However, more preferably, the parameters relevant to the crystal specimens are stored in a database on one data storage media, for example a hard drive, with an index or record pointer to the appropriate image stored on the removable drive with the optical images. In this fashion, a large

database of the various crystal parameters may be maintained and analyzed relative to each other with access to the optical images always available as required or desired.

Following acquisition of the optical image as well as the other crystal parameters, the computer is programmed to analyze the optical image for the presence and count of protein crystals. As a part of the evaluation, the computer program identifies the edges of the crystal, filling in any gaps of the edge where necessary, and then analyzes the resulting data for its perimeter symmetry and roughness, straightness, crystal size, presence or absence of defects and center of gravity. The crystal rating is then stored in the data base.

Preferably, the present invention utilizes a fast T-squared filer during its analysis of the optical image in order to grade the crystal. Alternatively, the present invention utilizes a 3x3 edge detection filter during its analysis of the optical image in order to identify the crystal edges, then the image is converted to a binary image with a threshold of approximately 40 on a scale of 0 to 255 to reduce image artifacts. The size of the crystal is determined via perimeter connectivity analysis. Objects with a small perimeter are excluded. The net resulting image is analyzed for its roughness which is a measure of the perimeter divided by the convex perimeter. This metric is used to isolate the crystal from the drop boundary and other artifacts. The center of gravity is calculated on the remaining data to pinpoint the crystals.

Alternatively, other methods may also be used to grade the crystal based upon metrics such as edge straightness, aspect ratio, surface clarity, polygon formation, color etc. These methods include the use of traditional spatial filters such as highpass, lowpass, Butterworth, homomorphic, Sobel, Laplacian, etc. Probabilistic restoration such as least mean square (Wiener) filters, fast T-squared filters, spatial transformations, frequency transformations, etc. can be used. Edge linking and boundary detection using Hough Transforms, "Line-filler" filters, thresholding, etc. can be used. Representation and description using Fourier descriptors, topological descriptors, texture descriptors, statistical descriptors, moments, mathematical morphological descriptors, etc. can be used. Recognition

using minimum distance classifiers, correlation classifiers, statistical classifiers, Bayesian classifiers, neural networks, genetic algorithms, etc. can be used.

Brief Description of the Drawing

A better understanding of the present invention will be had upon reference
5 to the following detailed description when read in conjunction with the accompanying drawing, wherein like reference characters refer to like parts throughout the several views, and in which:

10 FIG. 1 is a block diagrammatic view illustrating a preferred embodiment of the present invention;

FIG. 2 is an exemplary image of a crystal; and

FIGS. 3 through 6 are flow charts illustrating the operation of the system of the present invention.

Detailed Description of a Preferred Embodiment of the Present Invention

15 With reference first to FIG. 1, a block diagrammatic view of a preferred embodiment of the system of the present invention is there shown and comprises a video camera 12 which, upon activation, generates a signal on its output line 10 representative of the optical image in its focal window 16. A tray 18 containing the crystals under examination is positioned within the focal window 16.

20 Although the tray 18 can be positioned manually within the focal window 16 of the camera 12, in the preferred embodiment of the invention, preferably a plurality of trays 18, e.g. two trays, are positioned on a movable stage at a time and each tray contains twenty-four to ninety-six wells while a motorized mechanism 20 is used to control the position of the stage 22. A central processing unit or computer 24, under program control, then controls the activation of the motorized mechanism 20 by generating appropriate signals on line 25 and thus controls the position of the stage 22. In doing so, the motorized mechanism 20 automatically sequentially positions the individual wells 18 within the focal window 16 of the camera 12 so that an optical image of each well 18 is acquired.

In one embodiment, the computer 24 can control the ability of the system to focus, as well as zoom in/out on the specimen, and then store this information.

Once the motorized mechanism 20 has positioned a desired well 18 in the focal window 16, the central processing unit 24 generates an output signal on its output line 26 to the camera 12 in order to activate the camera 12 to produce signals on its output line 14 representative of the crystal image. These output signals are fed as input signals to the central processing unit 24 either directly via a port on the central processing unit 24 or via an appropriate input device 28, such as an appropriate video card.

10 In order to ensure even illumination of the crystal specimen during the image acquisition by the camera 12, preferably a fiber optic lighting system 30 is utilized as a back light under the tray 16. In one embodiment, the computer 24 can control the intensity of the lighting system 30, as well as the presence of light. Additionally, the computer 24 can also control polarizing means, such as the angle 15 of polarization. Other illumination means, however, may alternatively be used.

Following acquisition of the output signal from the video camera 12, the central processing unit 24 stores the image on data storage media using a storage device 32. Since a digital representation of an optical image typically consumes a relatively great amount of memory, e.g. one megabyte, the data storage device 20 32 is preferably a removable data storage media such as a JAZZ™ drive, ZIP™ drive or CD ROM, etc. Other types of media, including other types of removable data storage media, may alternatively be used.

25 During the data acquisition of the image by the central processing unit 24, the technician also utilizes a keyboard 34 or other data input equipment together with a video monitor 36 to input data pertinent to the particular crystal specimen. Such data input can include, for example, the pH of the specimen, temperature of the specimen, protein type of the specimen, detergents, additives, preservatives, reservoir buffer and associated variables of concentration, pH and volume, notes, score, drop descriptor, etc. Furthermore, in order to facilitate the entry of these 30 additional parameters by the technician, the central processing unit 24 preferably utilizes software templates displayed on the video monitor 36 to eliminate, or at

least minimize, the entry of redundant or repetitive information by the technician. Examples of such are included in Appendix A, incorporated herein.

5 The various parameters for each crystal specimen are also stored on data storage media, such as a hard drive 40, in a database and preferably in a relational database. Since the various parameters pertinent to the crystal specimens consume considerably less storage space than the optical images themselves, preferably the parameters are stored on the hard drive 40 separate from the optical images stored by the data storage device 32. In doing so, the parameters stored on the hard drive 40 would include an index or pointer to the optical images stored by the data storage media unit 32 so that the various crystal parameters stored in the database 10 may be easily correlated whenever desired to the optical images.

15 The storage of the optical images of the crystals on data storage media as well as the storage of the crystal parameters in a database, such as a relational database, provide substantial advantages over the previously known photography and handwritten notes currently utilized by technicians. Perhaps most importantly, the use of a relational database allows the data to be analyzed, sorted, correlated and otherwise manipulated under software control by the central processing unit 24. The storage of the optical images on data storage media also provides a permanent record of the images without the previously known degradation and possible damage of photographs.

20 With reference now to FIG. 2, an exemplary image of a crystal stored by the central processing unit on data storage media is there shown. The crystal 150 includes a plurality of edges 152 having a certain degree of straightness. Additionally, the edges 152 of the image of the crystal 150 may include gaps 154 due to lighting deficiencies during the image acquisition, deficiencies of the camera 12, or for other reasons.

25 With reference now to FIG. 3, a flow chart illustrating the operation of the present invention is there shown. At step 50, the program is initialized and the central processing unit 24 generates signals on its output line 25 to the motorized mechanism 20 (FIG. 1) in order to move the stage 22 to position the first crystal tray well 18 within the focal window 16 of the video camera 12. Step 50 then branches to step 52. At step 52, the central processing unit 24 generates an output

signal on line 26 to activate the video camera 12 to acquire the optical image and to input the optical image from the camera output line 10 of the camera 12 to the central processing unit 24. Step 52 then branches to step 54.

At step 54, the central processing unit 24 stores the optical image via the data storage device 32 (FIG. 1). Optionally, appropriate data compression software may be utilized to minimize the storage required by the optical image of the crystal storage. Likewise, optionally digital filtering algorithms may be also utilized to remove extraneous noise and otherwise enhance the digital image prior to storage on the data storage media by the storage device 32 or 40.

Concurrently with storing the optical image of the crystal on data storage media, the technician inputs various relevant parameters, such as pH, temperature, crystal growth duration, protein type, et cetera pertinent to the crystal under examination via the keyboard 34 or other input device. Preferably, the central processing unit 24 under software control provides appropriate templates to the technician replicating various parameters common to the various specimens in the trays 18 on the stage 22. In doing so, the entry of redundant or duplicative information by the technician between crystal specimens is minimized which minimizes not only the labor required from the lab technician, but also the entry of errors through human technician error.

The various crystal parameters entered by the technician are also stored at step 54 by the central processing unit 24 on the data storage media 32 or 40 (FIG. 1). Stored concurrently with the crystal parameters on the data storage media 32 or 40 is an index or pointer which correlates the stored crystal parameters to the stored image on data storage media by the data storage device 32.

Step 54 then branches to step 56 whereupon the central processing unit 24 generates output signals on its output line 25 to activate the motorized staged mechanism 20 to move the stage 22 to position the next well in the focal window 16 of the camera 12. Step 56 then branches to step 52 where steps 52 and 54 are reiteratively executed until an optical image and crystal parameters for each of the wells 18 on the stage 22 have been acquired and stored as previously described.

With reference now to FIGS. 4 and 5, following the acquisition and storage of the images and crystal parameters, step 58 is then executed to analyze the stored image. Step 58 is illustrated in greater detail in FIG. 5 and FIG. 6.

With reference then to FIG. 5, during the analysis of the stored image, the 5 program at step 60 executes a fast T-squared filter in order to locate and identify the edges of the crystal image. The fast T-squared filter is essentially a local digital filter which looks for a line defined as a set of co-linear pixels having a mean value different from the mean value of adjacent pixels on both sides of the line. Preferably, the fast T-squared filter at step 60 computes the two sample T-statistic with a null value of 0.

10 Preferably, the T-squared filter utilizes integer mathematics to minimize computing time required by the central processing unit. The T-squared filter at step 60 is also preferably a variable width T-filter which identifies lines having variable widths, e.g. a width varying in size from one pixel to five pixels. Step 60 then branches to step 62.

15 At step 62 a low pass filter is applied to the image to remove extraneous noise. Step 62 then branches to step 64.

20 At step 64, the program "fills" digitally any gaps, such as the gap 54 in FIG. 2, which may be present in the digitized image. Thus, following step 64, the program has identified not only the lines of the crystal, but also eliminated extraneous noise in the crystal image as well as completed any missing segments of the crystal edges due to deficiency of the camera, lighting or the like. Step 64 then branches to step 66 (FIG. 4).

25 At step 66, the program "scores" or evaluates the crystal images and then stores the score or evaluation as one of the crystal parameters on the storage device 40. Such scoring includes examination and evaluation of the straightness of the crystal edges, the presence or absence of defects in the crystal, fractures in the crystal and the like.

30 With reference then to FIG. 6, during the analysis of the stored image, the computer is programmed to analyze the optical image for the presence and count of protein crystals. As a part of the evaluation, the computer program identifies

the edges of the crystal and then analyzes the resulting data for its perimeter symmetry and roughness and center of gravity.

Preferably, the present invention utilizes a 3x3 edge detection filter during its analysis of the optical image in order to identify the crystal edges, then the 5 image is converted to a binary image with a threshold of approximately 40 on a scale of 0 to 255 to reduce image artifacts. The size of the crystal is determined via perimeter connectivity analysis. Objects with a small perimeter are excluded. The net resulting image is analyzed for its roughness which is a measure of the perimeter divided by the convex perimeter. This metric is used to isolate the 10 crystal from the drop boundary and other artifacts. The center of gravity is calculated on the remaining data to pinpoint the crystals.

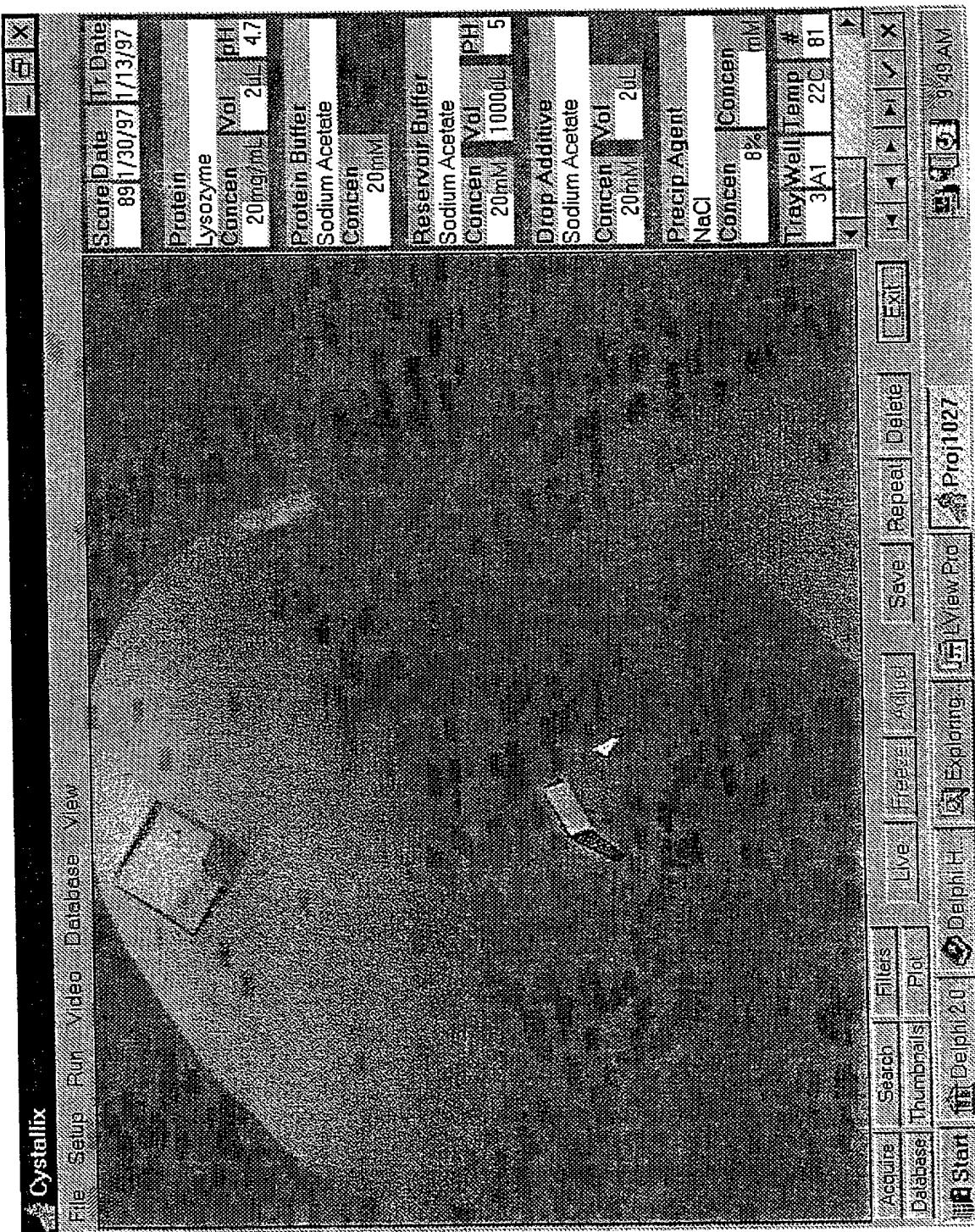
From the foregoing, it can be seen that the present invention provides a system for the automatic acquisition, storage and evaluation of crystal specimens under computer control. The use of software templates by the operator to 15 minimize the entry of duplicative or repetitive information further enhances not only the reliability of the data inputted into the system, but also minimizes technician labor.

A still further advantage of the present invention is the storage of the crystal parameters in a relational database which enables the manipulation and evaluation (i.e., experimental history, archiving, trend analysis, etc.) of the crystal 20 parameters under computer control utilizing search and 3-D plotting capabilities. The storage of the crystal images in removable data storage media separate from the storage of the crystal parameters also ensures that a large number of crystal parameters can be stored in the relational database with access to the crystal 25 images always available.

Having described our invention, however, many modifications thereto will be come apparent to those skilled in the art to which it pertains without deviation from the spirit of the invention as defined by the scope of the appended claims.

We claim:

APPENDIX A



Index	Protein	Protein Concentration	Protein Volume	Protein Molar	Protein Buffer
75	Lysozyme	36	2	4.7	Sodium Acetate
76	Lysozyme	36	2	4.7	Sodium Acetate
77	Lysozyme	36	2	4.7	Sodium Acetate
78	Lysozyme	36	2	4.7	Sodium Acetate
79	Lysozyme	36	2	4.7	Sodium Acetate
80	Lysozyme	36	2	4.7	Sodium Acetate
81	Lysozyme	36	2	4.7	Sodium Acetate
82	Lysozyme	36	2	4.7	Sodium Acetate

Score	Try	Yield	Try Date	Entity Date	Temp	Index
89	3	A6	1/13/97	1/30/97	22	79

Protein	Concentration	Volume	pH
Lysozyme	36 mg/ml	2.0 L	4.7

Protein Buffer	Concentration
Sodium Acetate	20 mM

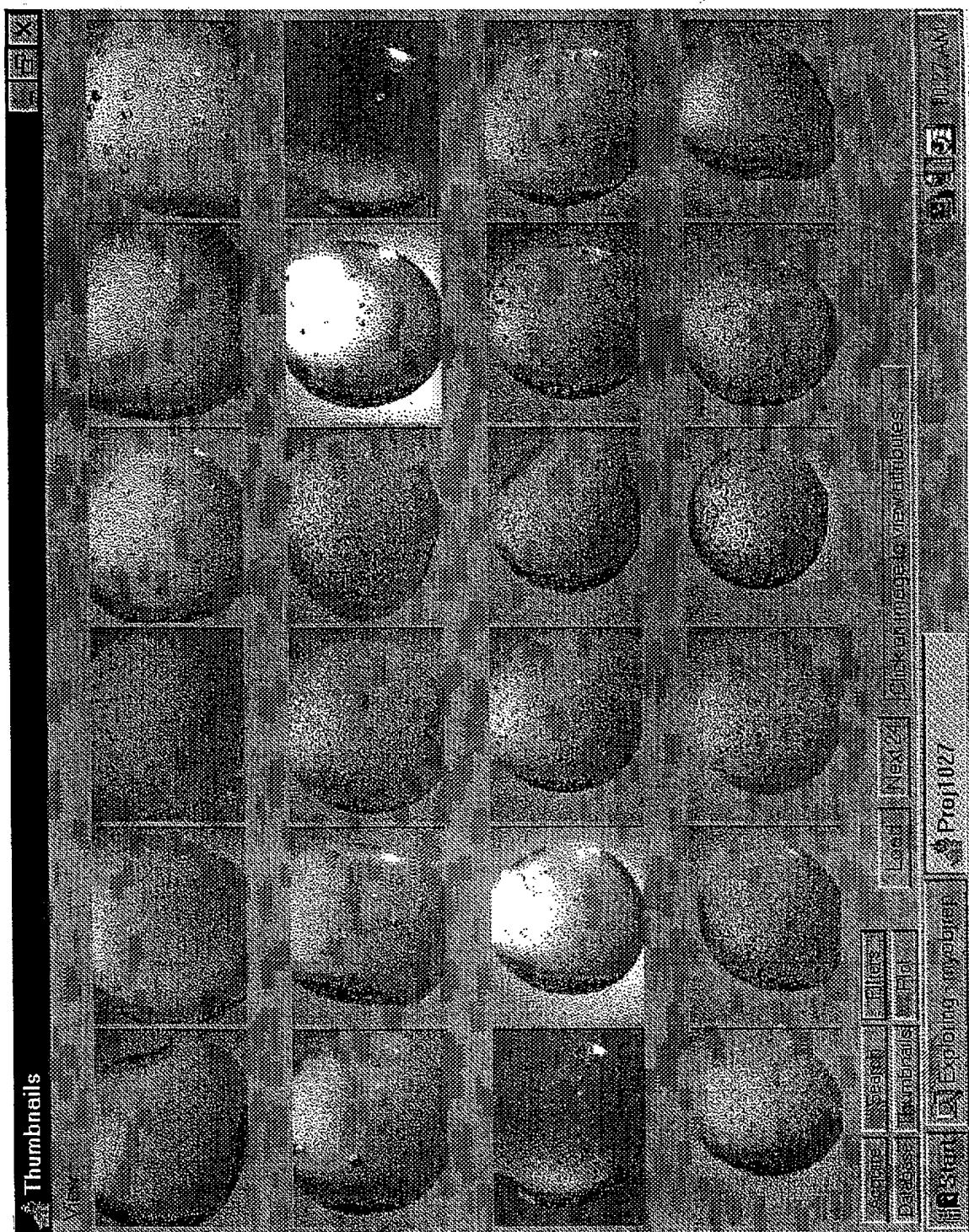
Reservoir Buffer	Concentration	Volume	pH
Sodium Acetate	20 mM	1.0 L	5

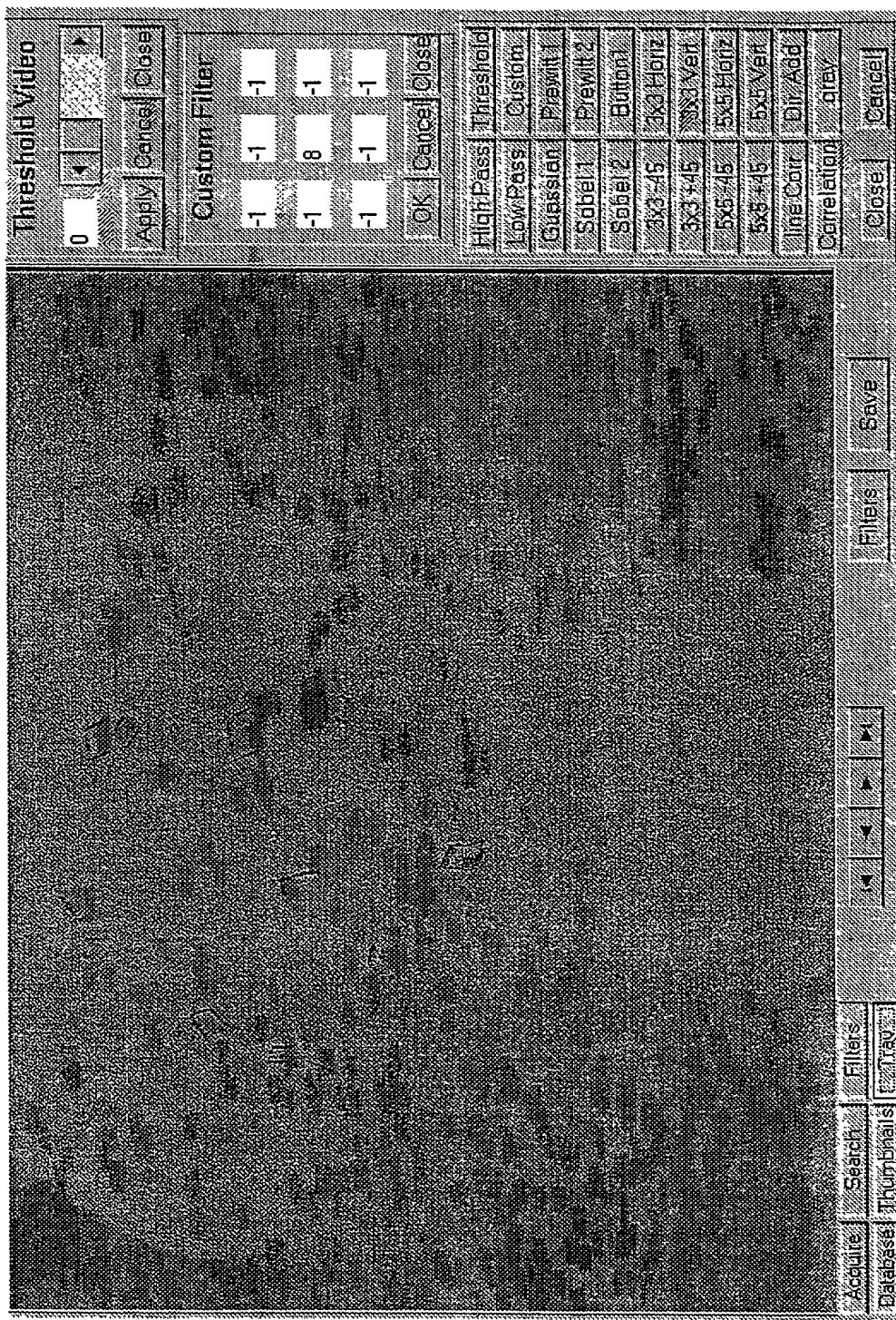
Dye Additive	Concentration
Sodium Acetate	20 mM

Preserving Agent	Concentration
NaCl	8 %

Acidifier	Search	Editor
Database	Thumbnails	Plot

Start	Delphi 20	Delphi 14	View Pro	Explore	PPF	Project	Team
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Claims

1 1. A system for utilizing a digital computer to evaluate microscopic
2 details of specimens, e.g. crystals, comprising:

3 a camera which generates an output signal representative of an image
4 positioned in a focal plane of the camera,

5 a tray which positions a specimen in said focal plane,

6 a computer processing unit having a persistent storage device, which
7 computer processing unit acquires said output signal from said camera,

8 said computer processing unit being programmed to evaluate said stored
9 image from said camera and for generating a result signal representative thereof,

10 said computer processing unit being programmed to store said result
11 signals in said persistent storage device.

1 2. The invention as defined in claim 1 wherein each said specimen
2 comprises at least one crystal.

1 3. The invention as defined in claim 2 wherein said computer
2 processing unit is programmed to count said crystals.

1 4. The invention as defined in claim 2 wherein said computer
2 processing unit is programmed to generate three-dimensional surface plots of
3 crystals within a database.

1 5. The invention as defined in claim 1 and comprising a movable
2 stage for automatically positioning said specimens in said focal plane.

1 6. The invention as defined in claim 2 and comprising a T-squared
2 filter to identify said crystals in each said specimen.

1 7. The invention as defined in claim 6 where said T-squared filter
2 comprises a software program executed by said computer processing unit.

1 8. The invention as defined in claim 5 wherein said computer
2 processing unit generates output signals to control the movement of said movable
3 stage.

1 9. The invention as defined in claim 4 wherein data relating to said
2 crystal(s) is stored in the database.

1 10. The invention as defined in claim 1 and comprising a light source
2 directed to said specimen.

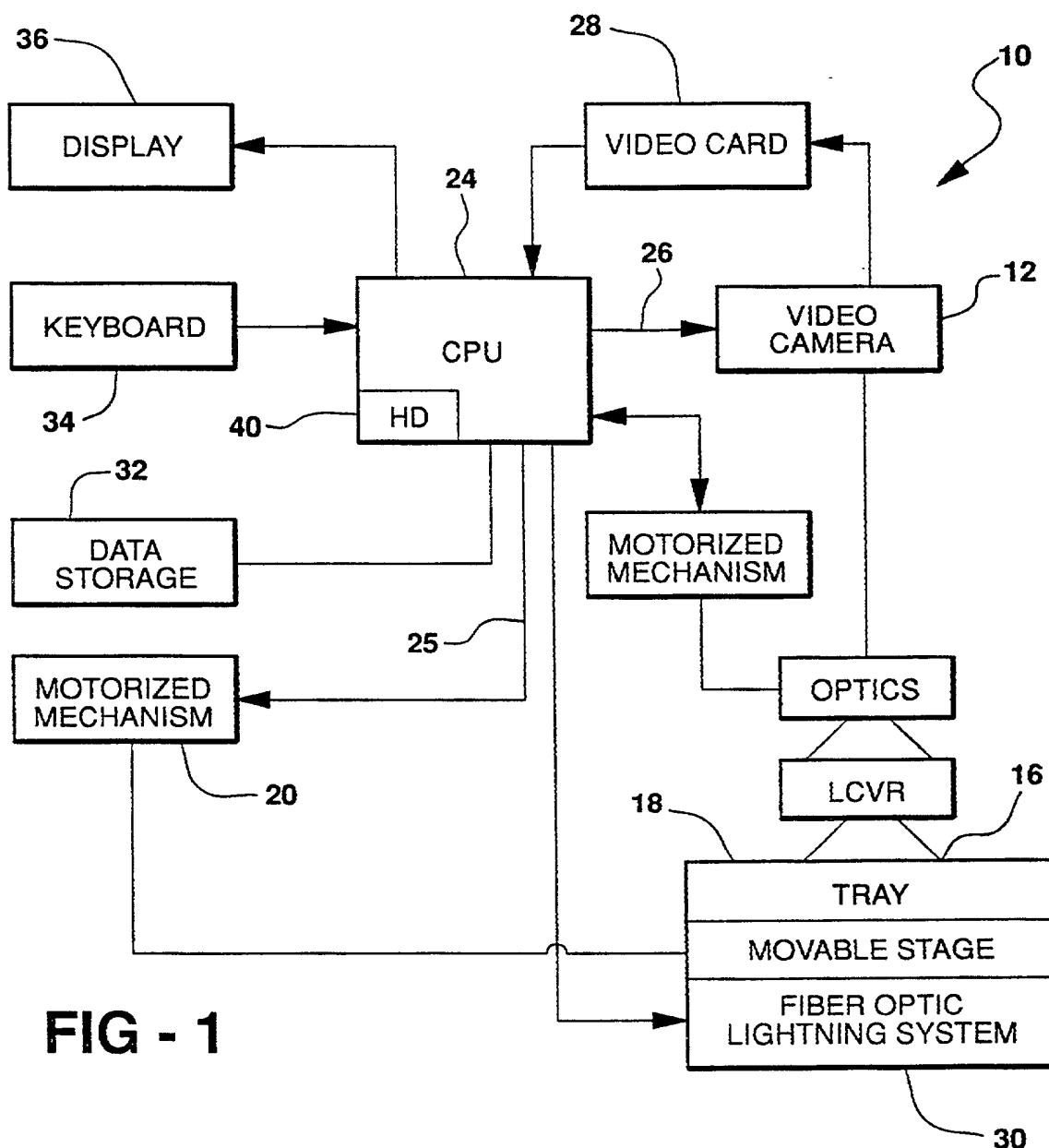
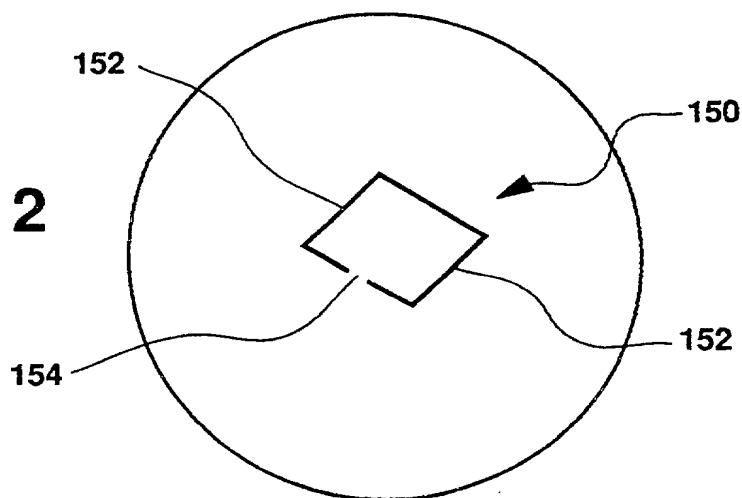
1 11. The invention as defined in claim 10 and comprising an optical
2 fiber extending between said light source and said specimen.

1 12. The invention as defined in claim 2 and comprising a computer
2 algorithm executed by said computer processing unit for rating said crystals with
3 respect to predetermined standards.

1 13. The invention as defined in claim 2 and comprising a computer
2 algorithm executed by said computer processing unit for simulating edges of
3 crystals missing in said image generated by said camera.

1 14. The invention as defined in claim 2 wherein said computer
2 processing unit is programmed to determine crystal size by determination of the
3 length of the perimeter of said crystals.

AB 2/21/2018

**FIG - 1****FIG - 2**

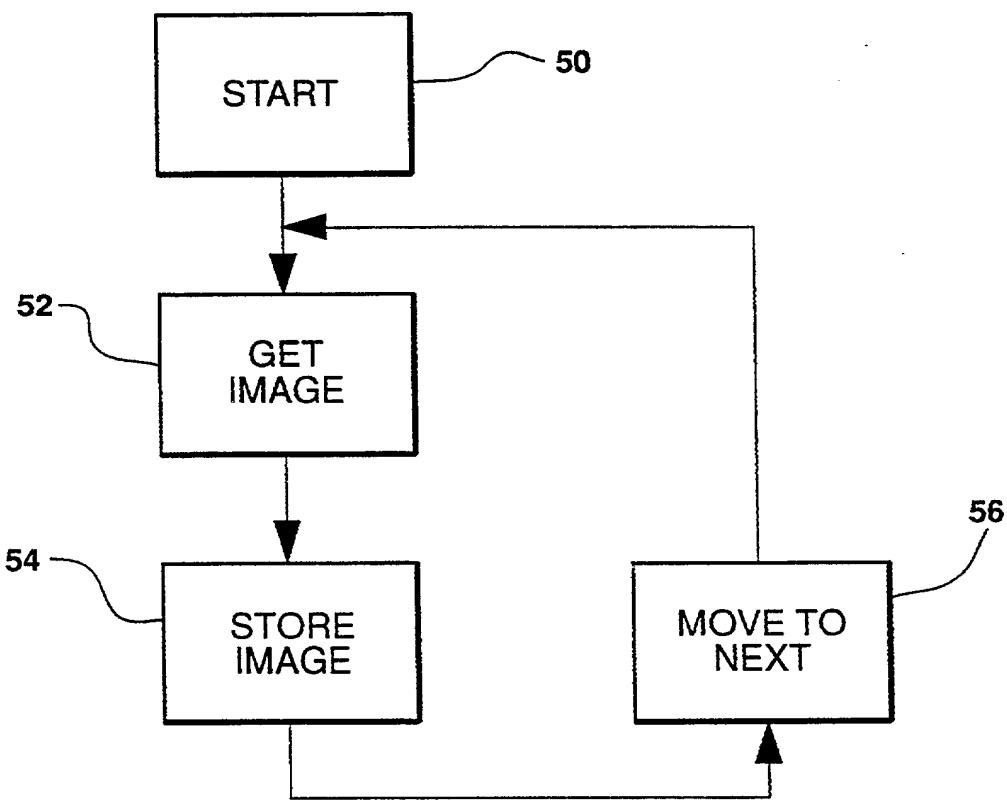


FIG - 3

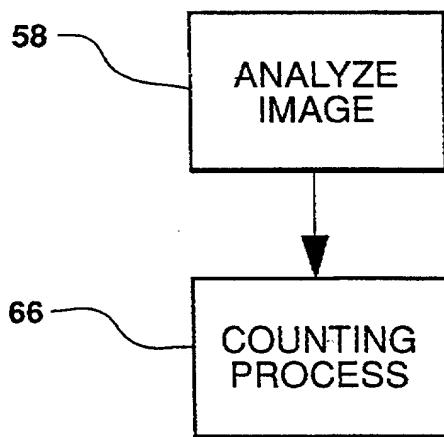
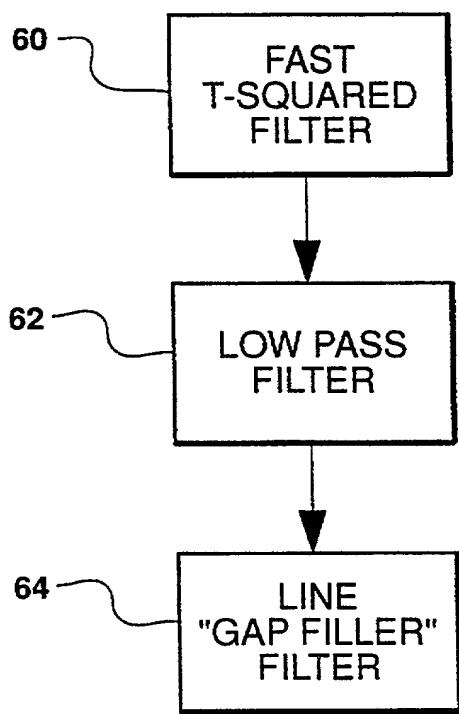
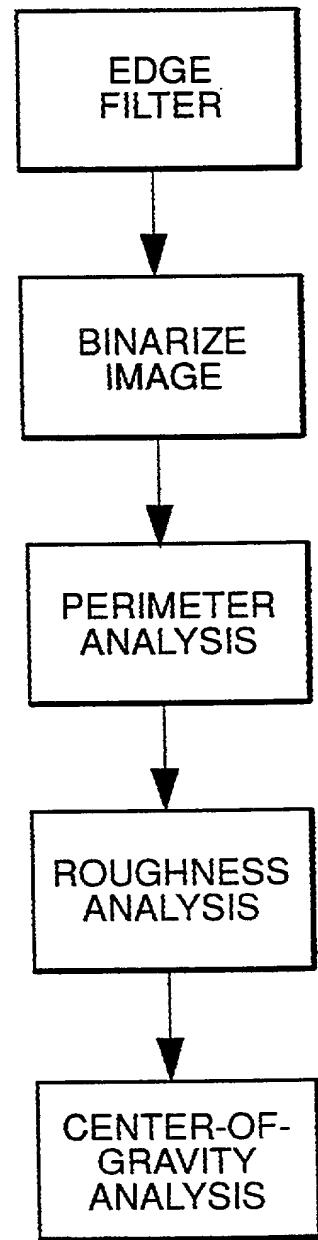


FIG - 4

**FIG - 5****FIG - 6**

Docket No.
DSI-10002/22

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD FOR ACQUIRING, STORING AND ANALYZING CRYSTAL IMAGES

the specification of which

(check one)

is attached hereto.

was filed on January 14, 2000 as United States Application No. or PCT International

Application Number 09/462,950

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

(Number)

(Country)

(Day/Month/Year Filed)

(Number)

(Country)

(Day/Month/Year Filed)

(Number)

(Country)

(Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

60/052,902
(Application Serial No.)

7/16/97

(Filing Date)

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/US98/14776
(Application Serial No.)

7/16/98

(Filing Date)

Abandoned

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)

(Status)
(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (list name and registration number)

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